

Superior Colliculus Lesions and Flash
Evoked Potentials from Rat Cortex

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16. ABSTRACT It is generally assumed that the primary response of the rat flash evoked potential (FEP) is activated by a retino-geniculate pathway, and that the second response reflects input to the cortex by way of the superior colliculus (SC) or other brainstem structures. In the present study, Male Long Evans rats were implanted with monopolar screw electrodes over the left visual cortex, and a pair of monopolar depth electrodes in each SC, which were used to produce electrolytic lesions. One half of the animals did not receive the electrolytic treatment (controls). FEB waveforms were obtained from all animals prior to treatment, and 2 and 5 days after treatment. Histological analysis was performed to verify electrode placement and determine lesion size. Electrolytic lesions which resulted in massive destruction of the SC produced no decrement in any portion of the rat FEP, but did produce an increase in amplitude of the N2P3 component. The data show that the secondary response of the rat FEP is not generated by SC, but that SC may modulate amplitude of the response.		
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Superior Colliculus Lesions and Flash Evoked Potentials From Rat Cortex¹

MARTHA I. BARNES AND ROBERT S. DYER²

Neurotoxicology Division, U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

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BARNES, M. I. AND R. S. DYER. *Super colliculus lesions and flash evoked potentials from rat cortex*. BRAIN RES BULL 16(2) 225-230, 1986.—It is generally assumed that the primary response of the rat flash evoked potential (FEP) is activated by a retino-geniculate path, and that the secondary response reflects input to the cortex by way of the superior colliculus (SC) or other brainstem structures. In the present study, male Long-Evans rats were implanted with monopolar screw electrodes placed over the left visual cortex, and a pair of twisted monopolar depth electrodes, which were used to produce electrolytic lesions, were placed in each SC. One half of the animals did not receive the electrolytic treatment (controls). FEP waveforms were obtained from all animals prior to treatment, and 2 and 5 days after treatment. Histological analysis was performed to verify electrode placement and determine lesion size. Electrolytic lesions resulting in massive destruction of the SC produced no decrement in any portion of the rat FEP but did produce an increase in amplitude of the N2P3 component. The data show that the secondary response is not generated by SC in rats, but that SC may modulate amplitude of the response.

Superior colliculus lesions Rat visual cortex Visual evoked potentials Flash evoked potentials

THE waveform of the flash evoked cortical potential (FEP) recorded in rats has been well described (e.g., [2,3]). The FEP is composed of a primary response (peaks P1, N1 and P2), mediated through the retino-geniculo-cortical pathway [1, 6, 10], a secondary response (peaks P2-N2-P3), which is thought to be mediated by indirect visual pathways [1, 3, 10], and a slow wave (peak N3), which has been described as phasic thalamocortical activity [1] possibly modulated by superior colliculus [2]. Although the anatomical and physiological evidence for the origin of peaks P1N1 and N3 is fairly strong [1], evidence for the anatomical and physiological basis of peaks P2-N2-P3 is not. The most compelling case has been made by Creel *et al.* [3], who pointed out that these peaks respond to changes in illumination and anesthesia in a manner similar to the "Type II" responses described by Torres and Warner [12] in rats, which are eliminated by transection at the level of the colliculi. In the view of Creel *et al.* [3] this secondary response is a geniculostriate response which is so heavily modulated that its appearance is dependent upon connections of the superior colliculus with brainstem and diffuse thalamic projections.

Although the FEP is being used increasingly as a diagnostic tool, interpretation of results is limited by uncertainties as to the generators of the peaks (e.g., [4]). Clearly knowing elements that contribute to each peak will simplify

interpretation of amplitude and latency changes resulting from neurological and toxicological insult. We decided to test the hypothesis that the superior colliculus (SC) is involved in the P2N2P3 wave recorded from rats. In particular, this study was designed to investigate the effect of bilateral SC lesions on FEPs recorded from male, Long-Evans hooded rats.

METHOD

Sixty-seven male Long-Evans hooded rats, age 60 days, were obtained from the Charles River Breeding Co., housed individually in plastic cages with woodchip bedding, and maintained on a 12:12 light-dark cycle with ad lib access to water and standard laboratory Purina Rat Chow. Upon attaining a weight of 375 grams, the animals were implanted with electrodes for recording and producing lesions.

For electrode implantation, animals were anesthetized intraperitoneally with 0.37 ml of Chloropent (Fort Dodge Lab, Inc.) per 0.1 kg of body weight. Supplemental doses were given as needed. Each animal was then placed in a stereotaxic frame with the incisor bar set at 5.0 mm above interaural zero [9], and implanted with 00-90×1/16 inch stainless steel skull screws for recording the FEP. Ground and reference screws were placed in the left and right side of the

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²Requests for reprints should be addressed to Dr. R. S. Dyer, Neurotoxicology Division (MD-74B), U.S. Environmental Protection Agency, Research Triangle Park, NC 27711.

skull, respectively, 2.0 mm anterior to Bregma and 2.0 mm lateral to the sagittal suture. The active recording electrode was placed over the left visual cortex, 6.0 mm posterior to Bregma and 4.0 mm lateral to the sagittal suture. Screw electrodes were connected to an Amphenol receptacle by 0.25 mm nichrome wire.

A pair of monopolar lesion electrodes made of 0.25 mm nichrome wire and insulated to within 1 mm of the tip, was placed in each superior colliculus at 5.0 mm and 6.0 mm posterior to Bregma, ± 1.5 mm lateral to the sagittal suture, and 4.4 mm below the dura. The lesion electrodes were attached to the Amphenol receptacle, and the entire assembly was cemented in place by dental acrylic. Upon completion of the surgery, the wound was sutured shut and each animal received 100,000 units of penicillin IM. A minimum recovery period of one week was allowed before recording.

Details of the recording chamber and methods have been published elsewhere [5]. Briefly, the animal was connected to the recording apparatus via an Amphenol plug and a Microdot mini-noise shielded cable. It was then placed in a mirrored recording chamber, which had the lamp from a Grass PS22 photostimulator mounted flush against one wall. The photostimulator was set at 16, which corresponds to a flash intensity of 4.53×10^2 lux-sec above background.

One half hour prior to recording, the pupils were dilated by topical application of 1.0% atropine. To allow for habituation to the chamber and flash, each animal was placed in the recording chamber approximately five min before the test session began. Strobe flashes were presented at 0.5 Hz for 45 sec prior to data collection. Subsequently, recordings were made using the same stimulus frequency and intensity.

During recording, the signal was amplified ($\times 10,000$), band-pass filtered (0.8 Hz to 10 kHz), sent to an oscilloscope for visual monitoring and to a PDP 11/70 computer for averaging the responses of 128 trials. The sampling rate was 2500 Hz with a post-stimulus epoch of 409.6 msec.

Averaged waveforms were displayed on a Tektronix 4054 graphic display computer terminal. Peaks were selected manually using a cursor, and peak-to-peak amplitudes and peak latencies for the three major waves in the cortical flash evoked potential were computed and displayed by the computer.

One week subsequent to surgery, a pre-treatment (prior to lesion) recording was obtained from all animals. Since the objective of this experiment was to determine alterations in the cortically recorded flash evoked potential as a consequence of lesion placement in the superior colliculus, it was necessary for the pre-treatment waveforms to exhibit normal characteristics. Due to natural variations between animals and abnormalities resulting from the implantation procedure, it is possible to obtain waveforms which deviate from "normal." Consequently, a subjective judgment of the "abnormality" of the pre-treatment waveform was rendered with regard to its morphology, latency, and amplitude. Only animals exhibiting a "normal" pre-treatment waveform were used for the experiments. Subjects were randomly assigned to either lesion or control groups, and the order of testing was randomly determined. All animals then received an in-

traperitoneal dose of Chloropent (0.35 ml/kg of body weight). Once anesthetized, animals assigned to the lesion group had two anodal, direct current lesions produced in each superior colliculus (one/electrode) using one channel of a Grass S88 stimulator and a PSU6 constant current converter. Lesions were produced using an array of cathodes. Each lesion electrode served as an active electrode (anode) with the three remaining lesion electrodes serving alternately as cathodes. The active electrode had a current of 1.4 mA passed between it and each cathode electrode for a duration of 20 seconds, resulting in a total current duration of 60 seconds at each lesion electrode. Preliminary data indicated that this procedure produced symmetrical lesions of appropriate dimensions.

Two and five days after placement of the lesions, the FEP for each animal was again recorded in accordance with the previously described procedure. Upon completion of the second post-treatment recording, each animal received an intraperitoneal dose of Chloropent (0.5 ml/kg of body weight). Once anesthetized, each animal was perfused intracardially with 10 ml isotonic buffered saline followed by a minimum of 30 ml phosphate buffered formalin. The electrodes were carefully removed from the brain, which was then removed from the cranium and placed in phosphate buffered formalin.

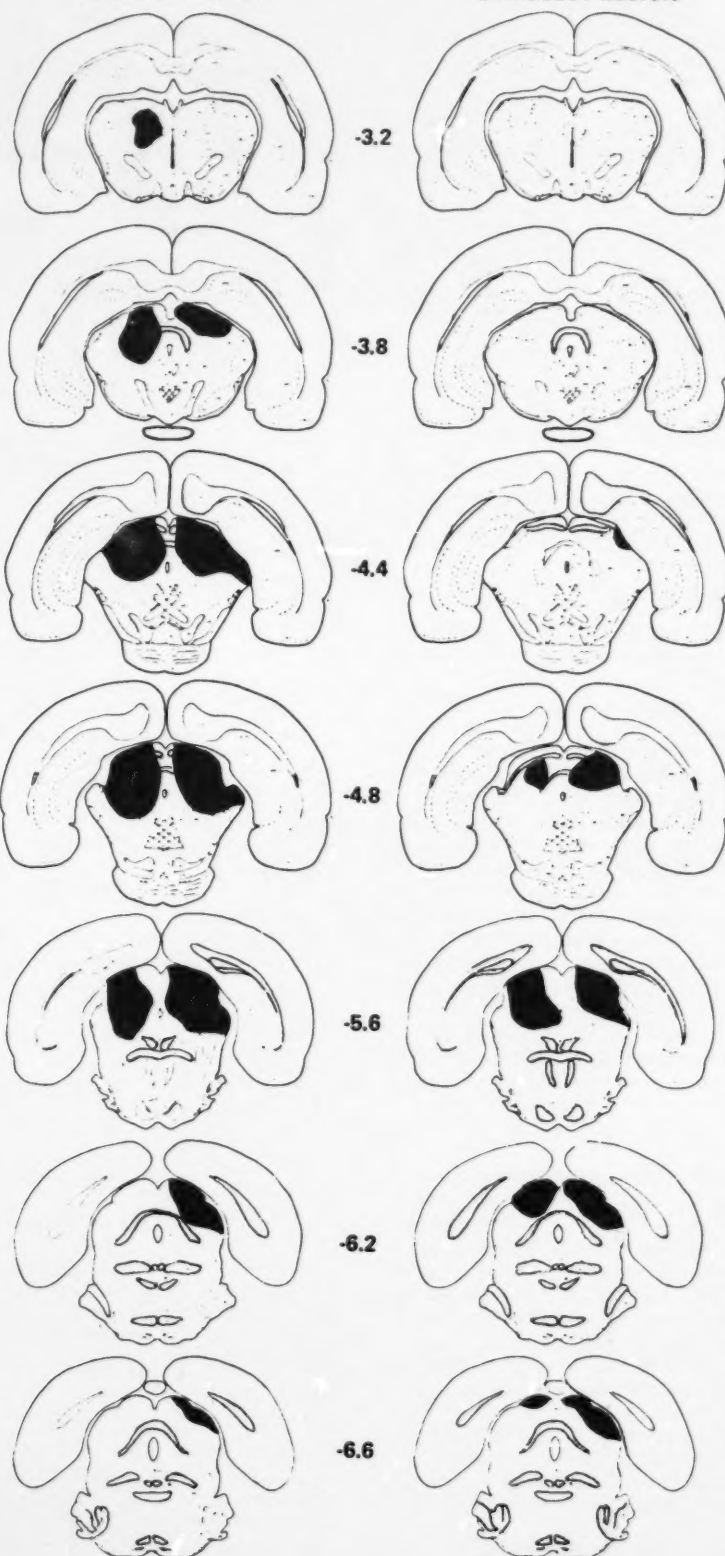
To confirm electrode placement and determine lesion size, each brain was blocked, embedded in paraffin, and serially sectioned in the coronal plane at 20μ thickness. Every fourth section through the block was mounted on a slide and stained with cresyl violet.

A minimum criterion for lesion size was established to allow exclusion of animals whose lesions were extremely small. This criterion was set according to the following hypothesis. Single unit mapping demonstrates point to point correspondence between the SC and the VC [11]. Since the flash evoked visual cortical potential is a summated response and there is no evidence to support one area of the SC having greater input into the VC than any other area of SC, equipotentiality of the SC (in terms of its contribution to cortically recorded flash evoked potentials) was assumed. It was, therefore, postulated that if the entire SC is needed to yield the VC P2-N2-P3 peak, then the destruction of x% will result in a decrement of the peak by x%. Previous data obtained in this lab indicates that with groups of the size used here, a change in P2N2 and N2P3 peak amplitudes of at least 25% is necessary to obtain statistically significant effects. The percentage destruction of tissue in control animals was added to the 25% destruction of the SC presumed to be necessary to produce a statistically significant effect in order to determine the minimal amount of SC destruction required for an animal's lesion to be considered successful.

To determine the lesion size, the cresyl violet stained sections were projected onto plain white paper. Using a polar planimeter, estimates of the size of the left SC, right SC, left lesion, right lesion, and the left and right lesion within its respective colliculus were obtained, and their respective areas were determined. The extent of SC destruction was calculated by determining the percent of the SC exhibit-

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FIG. 1. A series of coronal sections showing bilateral superior colliculus lesions from two animals demonstrating the largest (left) and smallest (right) percentage of superior colliculus destruction. The most anterior sections are displayed at the top with selected sections throughout the atlas of Pellegrino *et al.* [9]. The position, relative to Bregma, is indicated in millimeters between the corresponding sections.



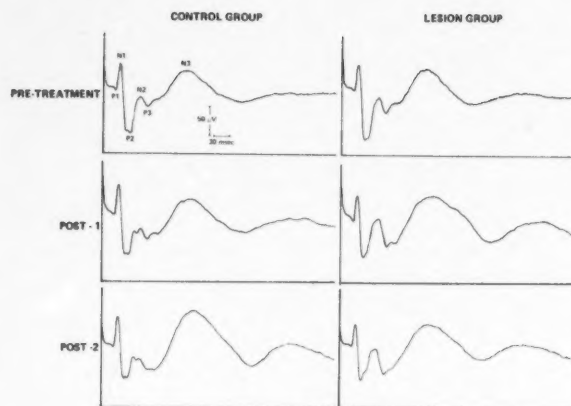


FIG. 2. Group averaged waveforms for control (left) and treated (right) groups. The top waveforms in each column were recorded prior to treatment. Post-1 waveforms were recorded 2 days after treatment, while Post-2 were recorded 5 days after treatment.

ing damage. Lesion quality was ascertained by dividing the area of the lesion within the colliculus by the area of the entire lesion.

A 2 (lesion condition) $\times$ 3 (time) factor repeated measures experimental design was used. The question being investigated was whether destruction of the SC would result in the loss of, or decrement in, the P2-N2-P3 peak. Therefore, a repeated measures multivariate analysis of variance (MANOVA) was run only on the five amplitudes (P1N1, N1P2, P2N2, N2P3, P3N3). The overall alpha level for the MANOVA was set at 0.05. The statistical design called for following a significant MANOVA with univariate ANOVAs. To retain an experiment-wise alpha of 0.05, 0.05 was divided among the dependent variables in a manner reflecting the a priori hypothesis under test. The p values assigned to P1N1, N1P2, P2N2, N2P3, P3N3 were 0.003, 0.003, 0.02, and 0.003 respectively, according to strategies described by Muller *et al.* [8]. For descriptive purposes, significant treatment $\times$ time interactions required subsequent Bonferroni-corrected " t " tests to pinpoint significant mean differences.

RESULTS

Of the 67 animals which were implanted for the superior colliculus lesion experiment, data were discarded from 10 animals which eventually died or which lost their head plugs. The data from another 4 animals were eliminated due to abnormal pre-lesion waveforms, and the data from 1 animal were discarded due to loss of tissue sections. Of the remaining 52 animals, 27 received the control treatment and 25 received the electrolytic lesion treatment.

Histological analysis indicated that the average amount of SC destruction in the control animals was 2.2%. Thus, a minimum of 27.2% SC destruction was necessary for an animal to be considered successfully treated. The range of SC destruction in the 25 treated animals was 34.2%–77.8%. Therefore, the data from all 25 treated and 27 control animals were subjected to the repeated measures multivariate analysis of variance.

Histological analysis confirmed that electrodes were

TABLE 1
PROBABILITIES ASSOCIATED WITH BETWEEN GROUP
 t -TEST COMPARISONS

Variable	Pre-treatment	2 Days Post-treatment	5 Days Post-treatment
N1P2	0.0663	0.1801	0.5582
N2P3	0.1471	0.0002*	0.0406

*=Significant following Bonferroni correction.

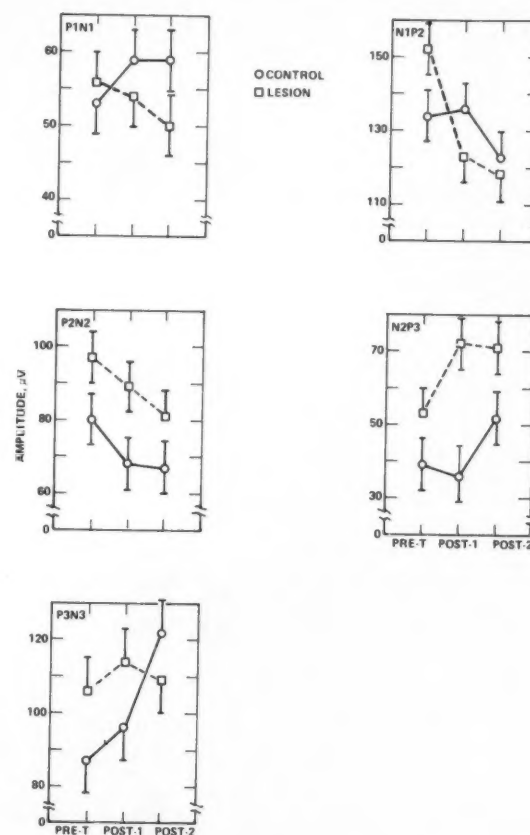


FIG. 3. Mean ($\pm$ SEM) amplitudes for the pre-treatment (Pre-T), first post-treatment (Post-1), and second post-treatment (Post-2) time points for each peak. Data from control rats are represented by solid lines, and data from the rats which received the lesion are represented by dotted lines.

accurately placed within the SC. Lesion quality for treated animals ranged from 0.604–0.874; i.e., 60.4–87.4% of the lesion was within the SC. The remaining damage was within structures located predominantly ventral and posterior to the SC, with the majority of the non-SC destruction located in

the reticular formation. No destruction was located within the lateral geniculate or other areas of the primary visual pathway. In Fig. 1, a series of sections shows the lesions from two animals, one with the largest and the other with the smallest lesions.

No lesion-related decrement was seen in any of the five peaks of the rat FEP. Peak N2P3 appeared to show an increase in amplitude. The group averaged waveforms from before and after treatment are shown in Fig. 2. These observations were supported by the statistical analysis. Since one of the time points was a pre-treatment value, only significant treatment $\times$ time effects were considered important. Only N1P2, $F(2,100)=11.13$, $p=0.0001$, and N2P3, $F(2,100)=4.60$, $p=0.0123$, demonstrated a significant treatment $\times$ time interaction. The probabilities associated with between group t -tests showed only the first post-treatment control vs. the first post-treatment test comparison for peak N2P3 to be significant, with $p=0.0002$ (Table 1). Thus, this effect appeared to be temporary. With regard to N1P2, neither of the between group t -test comparisons for recordings made after treatment approached significance, suggesting that the two groups were somewhat different at the outset of the experiment. Graphs of the means for each of the 5 amplitudes are shown in Fig. 3.

The possibility that the increased N2P3 amplitude observed in the rats with lesions reflected damage to the underlying reticular formation was examined by calculating Pearson rhos to assess the correlation between lesion size and location and N2P3 amplitudes. None of the correlation coefficients obtained by these calculations provided evidence of a relationship between damage to extra collicular areas and change in amplitude.

Behavioral tests were not performed on the animals in this study. However, certain behaviors were consistently observed. Animals exhibited circling behavior, increased excitability, and increased startle response and aggression upon approach to being handled, behaviors reported by others to follow SC lesions [7].

DISCUSSION

It was predicted that since the visual pathways in mammals have many similarities, and the effect of SC lesions in kittens [10] was the elimination of the secondary response (peaks P2-N2-P3), then the effect of similar lesions of the SC of Long-Evans rats would result in a similar decrement or loss of peaks P2-N2-P3 of the rat waveform. On the contrary, the results of this study showed no significant decrement in any of the five peaks comprising the FEP in rats.

The lesions produced in this study destroyed as much as 77.8% of the superior colliculus in the rat. Because the electrodes were placed centrally in the SC, the damage radiated

out from the center, resulting in extensive damage to all layers of the SC. Generally, destruction within the SC was more extensive in central and medial areas of the structure, with some sparing of the lateral edge. Occasionally some sparing of the stratum griseum superficiale and stratum opticum layers of the medial edge could be observed, but there was massive destruction of the lower gray layers which receive input from the superficial layers, and there was frequently considerable damage to the underlying reticular formation as well. Since SC impulses traveling to the reticular formation originate in the lower gray areas, they were effectively interrupted by the lesions. From this study, it does not appear likely that the cortical secondary response elicited in rats originates from a subcortical path through the SC and reticular formation.

The only significant alteration seen in the present study was a transient increase in amplitude of peak N2P3. The data for N2P3 also indicate a slight, though not statistically significant, difference between the treated and control groups at the initial test prior to the introduction of the treatment. To determine whether the difference between the control and treated groups during the pre-treatment test affected the differences between the two post-treatment tests, t -test comparisons were made on the amplitudes using difference scores which were obtained by subtracting the value of the pre-test from the corresponding post-treatment values. The comparison of the control vs. treated scores at the first post-treatment test for N2P3 yielded $p=0.0055$. The lesion-induced increase in the amplitude of N2P3, therefore, raises the possibilities that either SC or a path through the SC and reticular formation may modulate the secondary response in rats.

The present findings in unanesthetized rats are at odds with those of Rose and Lindsley [10], who studied FEPs elicited by flash stimulation in anesthetized kittens. Comparisons between these studies are confounded by differences in method, since Rose and Lindsley [10] used a less intense flash, anesthesia, and young cats. It is therefore not possible to state with certainty that differences between cats and rats account for the discrepancy.

This study has demonstrated that contrary to the generally accepted notion, the secondary visual evoked response recorded in rats is not generated by activity which originates at or below the level of the SC. Massive destruction of the SC resulted in no decrement in the secondary response or any other portion of the cortically recorded FEP. While the origin of this wave therefore remains to be determined, it seems likely that thalamocortical activity produces it, and that this activity can be modulated to some degree by mid-brain centers. This view, it must be noted, differs from that of Creel *et al.* [3] only in degree.

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